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14. ABSTRACT

The WRNMMC Clinical Breast Care Project/Winder Research Institute will continue to help lead the way in the 21st century in the crusade against breast disorders. The project will continue utilizing a multidisciplinary approach as the standard of care for treating breast diseases and breast cancer. This multidisciplinary model integrates prevention, screening, diagnosis, treatment and continuing care, but the project is further unique in the incorporation of advances in risk reduction, informatics, tissue banking and research. These efforts focus on decreasing the morbidity and mortality of breast cancer among American women.

15. SUBJECT TERMS
Biorepository, Biomedical Informatics, Focused Research, Breast Cancer Risk Reduction, Translational Clinical Care

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Breast Cancer Translational Research Center of Excellence FY12-15

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Breast Cancer Translational Research Center of Excellence FY12-15

Annual Report

COL Craig D. Shriver, M.D.; Principal Investigator and Director

1. INTRODUCTION

The Breast Cancer Translational Research Center of Excellence (BCTR-CoE) provides a multidisciplinary approach as the standard of care for treating breast diseases and breast cancer. This approach integrates prevention, screening, diagnosis, treatment and continuing care, incorporation of advances in risk reduction, biomedical informatics, tissue banking and translational research. The project is based on a discovery science paradigm, leveraging high-throughput molecular biology technology and our unique clinically well-characterized tissue repository with advances in biomedical informatics leading to hypothesis-generating discoveries that are then tested in hypothesis-driven experiments.

2. KEYWORDS: None

3. OVERALL PROJECT SUMMARY:

Objective/Hypothesis: Utilize and extend our unique DoD biorepository of well characterized biospecimens from a broad subset of patients with breast cancer and other breast diseases to broaden our knowledge of the etiology and pathology of breast disease specifically focused on breast cancers affecting the readiness of active duty women. Leverage the technological and information technology advances in genomic, proteomic, and total metabolomics research to further our understanding of breast cancer through discoveries in molecular biology, pathway analysis and systems biology that can be readily translated into the clinic.

Study Design: The project utilizes a multidisciplinary approach for researching breast diseases and breast cancer focused on the military at-risk population in order to enhance Readiness of The Total Force. This multidisciplinary model integrates prevention, screening, diagnosis, treatment and continuing care, but the project is further unique in the incorporation of advances in risk reduction, biomedical informatics, tissue banking and translational research. The project is based on a Discovery Science paradigm, leveraging high-throughput molecular biology technology and our unique clinically and pathologically well-characterized tissue repository with advances in biomedical informatics leading to hypothesis-generating discoveries that are then tested in hypothesis-driven experiments.

Background: Breast cancer is the most common non skin-related malignancy among women in the western world. It accounts for one-third of all cancers diagnosed. Age is the single most important risk factor for the development of breast cancer, as incidence and mortality both increase with age. However, a significant number of breast cancers are diagnosed among young women and this shift towards younger women developing breast cancer has increased in the past five years. Each year, over

10,000 new breast cancer cases are detected in women under the age of 40. Over 90% of these occur among women aged 30-39 years and 8 women per 10,000 in this age group die from breast cancer every year. Breast cancer is the single leading cause of death in women aged 40-49 years. Despite the low absolute risk of breast cancer in women under 40 years of age, the incidence is increasing in this age group. The incidence in younger women is probably underestimated based on the current understanding of the biology of breast cancer. The focus of the Breast Cancer Translational Research Center of Excellence (BCTR-CoE) is to work towards decreasing the morbidity and mortality of breast cancer among American women with a specific focus on the problem as it pertains to the active duty military population, an increasing number and proportion of which are female and are in this under-40 age group of increasing breast cancer development, risk, and poorer outcomes. As all jobs and positions in the military are now available to women including combat positions, the increasing incidence of breast cancer in younger (military-age) women and the increased lethality of that subtype of breast cancer, coupled with the military's critical reliance on a Total Force of all personnel inclusive of a high and increasing percentage female, demands a continued effort of the DoD through the BCTR-CoE to focus on surveillance, screening, early detection, curative treatments, and post-treatment Return To Duty Survivorship programs. The BCTR-COE has had a 14 year history of doing just that, and we are robustly moving into the future by targeting our valuable resources to the active duty military cancer problem, aligning ourselves with other DoD and federal agencies in order to increase efficiencies and allow best use of government funds, and ensuring we are in complete alignment with the DoD QUAD AIM with the central pillar of our efforts focused on READINESS of the Total Force.

Military Relevance: Breast cancer is the most common non-skin cancer in women. It is the single greatest cause of cancer deaths among women under 40, and is a significant cause of mortality for women in the United States Armed Forces. Breast cancer mortality among women <50 years accounts for >40% of years of life lost due to this disease. The economic, social and emotional costs to families are far greater when a young woman dies than when an older woman dies of breast cancer. The more aggressive nature of the disease in young patients along with the attendant costs underscores the importance of early detection of breast cancer in young women. Breast cancer is a curable disease if it is detected early; as such early detection is related to survivorship, cost of treatment and quality of life for the affected woman.

The majority (>90%) of women in active military service are < 40 years of age. The Department of Defense (DOD) with its high percentage (and increasing percentage, as all roles in the military are now open to all genders, including combat roles) of young women and its commitment to health care is particularly concerned about breast cancer. When discovered at a later stage, treatment of breast cancer is expensive, aggressive and results in considerable disruption to the woman's ability to contribute to the military and society. Cost and disruption to life are considerably less when the carcinoma is discovered at an earlier stage and therefore treatable with less invasive methods and curable in up to 90% of cases for Stage I disease. Furthermore, the DOD has a high percentage of African-American (~30%) and Hispanic (~10%) women. Death rates from breast cancer tend to be particularly high in these ethnic groups owing in part to later stage of detection and to the more aggressive nature of breast cancer in these groups.

The active duty military force is approximately 20% female. Most of these service members are in the age range (30-40 years) where routine screening for breast cancer consists only of clinical breast examination. Both mammography and clinical breast examination have a very poor accuracy in the

young active duty force in determining which breast abnormalities require treatment, and which are benign and can be left alone. The immense scale and impact of this problem for the military can be assessed by the fact that there were over 2,000 cases of breast cancer diagnosed in active duty service members over the last ten years (source: ACTURS DoD Tumor Registry data).

Furthermore, there were over 8,000 unnecessary breast biopsies done on active duty women during this time because it takes 4 breast biopsies of normal non-cancerous lesions to find each individual breast cancer. Hence, women often need to take lengthy amounts of time off from duty in order to undergo multiple tests leading up to the biopsy as well as time off from duty because of the biopsy itself. This translates into approximately 10,000 weeks, or 30 person-years, of time lost in the evaluation of normal, benign breast lesions in active duty service members. This would be unacceptable for any other healthcare issue, and should be so for this one. Unfortunately, at the present time there is no completely accurate screening tool currently available to diagnose breast cancer in the early, curable stages for women under the age of 40, who make up the vast majority of women in military uniform.

As indicated, approximately 20% of the active duty military force is female, most under the age of 50. Breast cancer strikes one in eight women in her lifetime, and there is a documented change in breast cancer incidence in recent years, such that breast cancer is being detected and diagnosed more often in younger women (under age 50), and the same is true in our military members. In the same way that diagnostic and therapeutic efforts through the military and US Army are carried out in infectious disease care and research, eg. Malaria, Typhoid, etc., so too must the military continue to address the effects of the scourge of breast cancer and breast diseases on the 20% of total active duty force who are women.

Moreover, CBCP, now the BCTR-COE, developed and to this day maintains the only specialty breast cancer evaluation and treatment center in the US Army, which is at the CBCP Comprehensive Breast Center at Walter Reed National Military Medical Center.

Additionally, ours is the only Army facility that financially supports direct genetic testing of active duty (all Services) women who are identified in our Center as being in a high risk category of carrying a BRCA genetic mutation, which when present can signify an up to 90% increased risk of breast cancer development, and for which we then deploy individualized cancer preventive therapies.

The BCTR-COE (CBCP) Breast Center is the Army-recognized and Military-recognized specialty referral center for tri-service active duty personnel from around the globe with medical disorders related to all breast diseases and breast cancer. CBCP Breast Center routinely cares for women on active duty Army from places such as the Middle East, Southwest Asia, OEF, Korea, Europe, and the Far East. CBCP at WRNMMC annually cares for over 7,500 patients.

Public Purpose: The BCTR-CoE is the continuation of the Clinical Breast Care Project (CBCP) that has been ongoing for 15 years. Its uniqueness and relevance has been attested to by numerous outside world-class cancer experts, from the innumerable public scientific and invited lecture presentations made by CBCP PI and investigators over the years, as well as by the extensive peer-reviewed publication record of CBCP researchers. The BCTR-COE has the world's largest biorepository of

highly-characterized and pristinely-collected specimens from breast patients made up of human breast tissues, lymph nodes, sentinel nodes, sera, bone marrow aspirates, cancers, benign tumors, and pre-malignant disease, which amounts in-total presently to **61,469 as of 23 August 2015**. This unique DoD resource, stored, maintained, tracked, and kept under strict QA in the CBCP-contracted repository at the Windber Research Institute since 2001, is used by both internal genomic and proteomic researchers, as well as for targeted collaborations with extramural collaborators from academia, governmental organizations, and corporate entities.

This biorepository is also unique in that its specimens are tightly coupled to highly-accurate clinical, demographic, and pathologic data collected from its originating patients through robust IRB-approved and fully HIPAA (Health Insurance Portability and Accountability Act)- compliant protocols that exceed all existing regulatory requirements for patient consent, privacy, and oversight.

The BCTR-COE has one of the few fully integrated genomic and proteomic molecular biology research programs in the nation devoted exclusively to research in breast diseases. We have an established track record of publication and scientific communication in this field.

The BCTR-COE has deployed a unique biomedical informatics data warehouse system that integrates clinical, pathologic, and molecular data on breast research subjects, allowing for a novel in-silico biology discovery platform.

The BCTR-COE is a true translational research-clinical care environment, where there actually exists an organizationally-driven and structured collaborative effort between basic scientists, clinical scientists, clinicians, nurses, patients, and multiple other personnel.

The BCTR-COE has successfully expanded to other clinical sites and has established other research collaborations with world-renowned lab researchers.

Specific Aims: This project is structured around two major themes for BCTR-COE research. Theme 1 focuses on breast cancer mechanistic studies of clinically important questions, and Theme 2 focuses on therapy-relevant molecular studies of breast cancers. These themes inform research across the five BCTR-CoE pillars: *(1) Breast Cancer Risk Reduction; (2) Biorepository; (3) Focused Research; (4) Biomedical Informatics; and (5) Translational Clinical Care.*

Pillar Specific Goals, Objectives and Status for this annual period

I. Breast Cancer Risk Reduction:

<u>Objectives</u>:

- To collect data on all female patients 18 and older who present to the CBCP Breast Center of Excellence at Murtha Cancer Center at Walter Reed National Military Medical Center - Bethesda and are found to be at an increased or elevated risk for developing breast cancer.

- To utilize this database to analyze the diagnosis, treatment, and treatment outcomes for patients found to be at an increased risk for developing breast cancer. Analysis includes but is not limited to: risk factors for developing breast cancer, effectiveness of various modalities of risk-reduction treatment (medical, surgical), and actual risk of developing cancer.

The Risk Reduction Clinic at WRNMMC is a multi-disciplinary program designed to identify, counsel and manage women at high risk for breast cancer. Patients receive an in-depth personal and family health history by a world renowned medical oncologist. **A total of 389 patients were managed; 310 patients were seen and 79 telephone consults were conducted at Walter Reed National Military Medical Center from 24 August 2014 – 23 August 2015.**

Current research shows there are risk factors that may influence the development of breast cancer. Identifying people with these risk factors and implementing closer surveillance and risk reduction techniques may detect cancer earlier. Earlier detection of breast cancer leads to better prognosis and outcomes. Calculations of risk are based on computer models extensively validated as accurate in identifying women at high risk.

Study Design and Methodology: Patients seen in the Comprehensive Breast Center at WRNMMC or at the JMBCC in Windber, PA were assessed for their risk of developing breast cancer by their history of LCIS or ADH or by applying the NCI Breast Cancer Risk Assessment Tool. Identified high-risk patients were referred to the CBCP Risk Reduction Clinic. Patients were confirmed to meet the inclusion criteria and consented to one of two core protocols. Information collected included the data contained on the enclosed database forms and from previous clinic visits. All applicable patients will be followed indefinitely according to the applicable protocol.

II. Biorepository:

- Continue to collect and store a broad spectrum of biospecimens from every patient undergoing a breast biopsy and/or breast surgery at WRNMMC, WMC, AAMC, and our affiliated hospitals that consent to participate in BCTR-COE IRB-approved protocols.
- Continue to collect and store biospecimens (blood) from women who are free of breast disease who consent to participate in BCTR-COE IRB-approved protocols to act as controls.
- Utilize the power of this extensive biorepository as a major resource for breast disease research.
- Leverage the BCTR-COE biorepository to maximize the utilization of the repository, with BCTR-COE leadership approval, for the overall benefit of breast cancer patients and research, as able and appropriate.
- Participate in national/international projects that can benefit from resources of the BCTR-COE biorepository.

Although there have been remarkable improvements in breast cancer diagnosis and management, most of the complex molecular mechanisms associated with the onset, progression and/or severity of breast cancer are still not well understood. As part of the BCTR-COE we carry out molecular, biochemical and histological analysis of breast tissue and/or blood and blood

components from breast cancer patients to provide insights into the molecular mechanisms that may be relevant in the development of breast cancer and breast diseases. To achieve this aim, a large supply and a wide variety of good quality tissue samples are needed. Unfortunately, good quality donor breast tissue is extremely scarce and when available is often not backed by a comprehensive medical history and/or is not a good representation of the target population or study area. The non-availability of a steady and consistent supply of good quality tissue limits the systematic analysis of tissues and negatively impacts the generation of biologically useful information in research laboratories and by extension negatively impacts new findings that benefit clinical practice. The objective of this project is therefore the acquisition and banking of breast tissue, lymph nodes, serum/plasma and other blood derivatives from informed and consenting donors.

Since the inceptions of the Clinical Breast Care Project the Biorepository Pillar has been critical to the success of the project. As we move forward into the establishment of the BCTR-COE it is important to look at the success of the biorepository and to understand the firm foundation that it has laid for building the Center of Excellence.

The charts below show the cumulative patient accrual into the CBCP protocols and total number of specimens stored in our biorepository since 2002. These patients, who have been recruited and consented into the CBCP protocols at WRAMC, WRNMMC, AAMC, JMBCC and other participating CBCP clinical intake sites are the foundations of the translational research that has occurred within the CBCP and which will continue in the BC-TRCOE. From these patients we have collected and stored in our biorepository over 61,469 biospecimens (**Figure BB-1**) donated by 7047 fully consented subjects to our IRB approved tissue and blood protocols. (**Figure BB-2**)

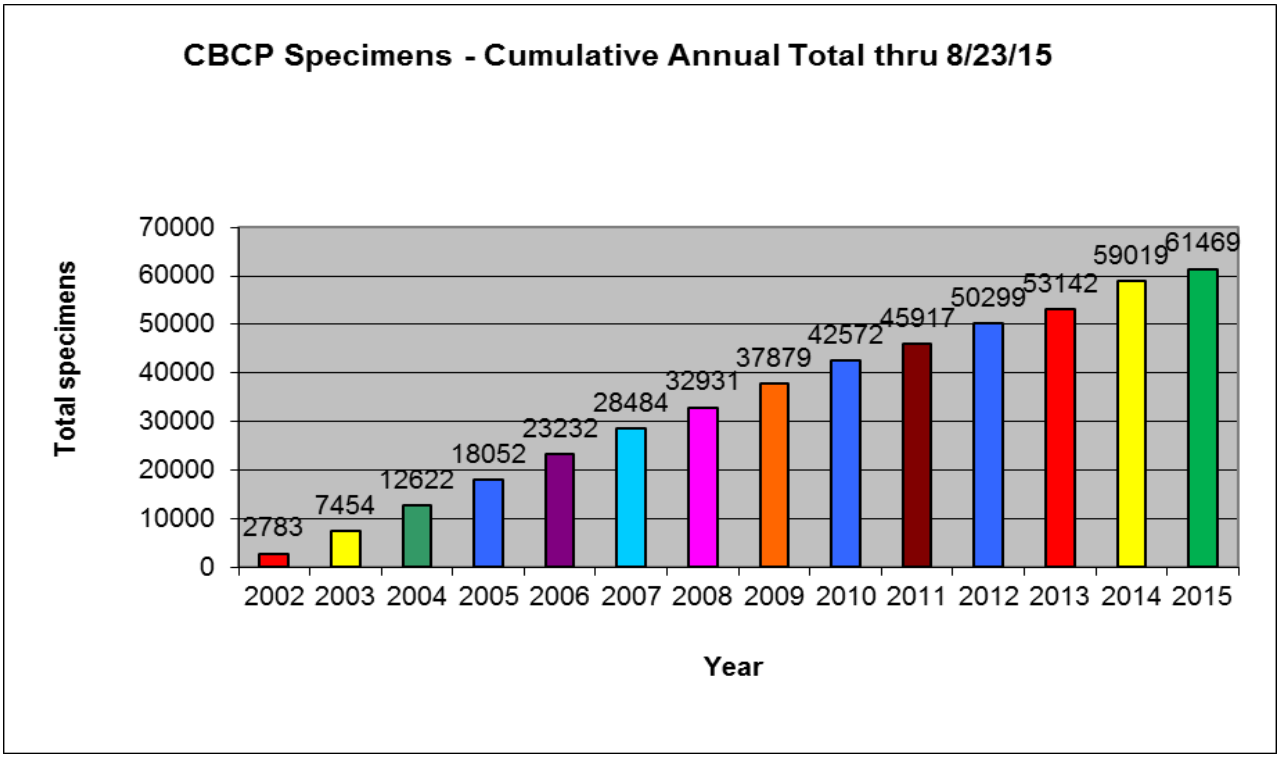


Figure BB-1 Total biospecimens collected and banked by the biorepository.

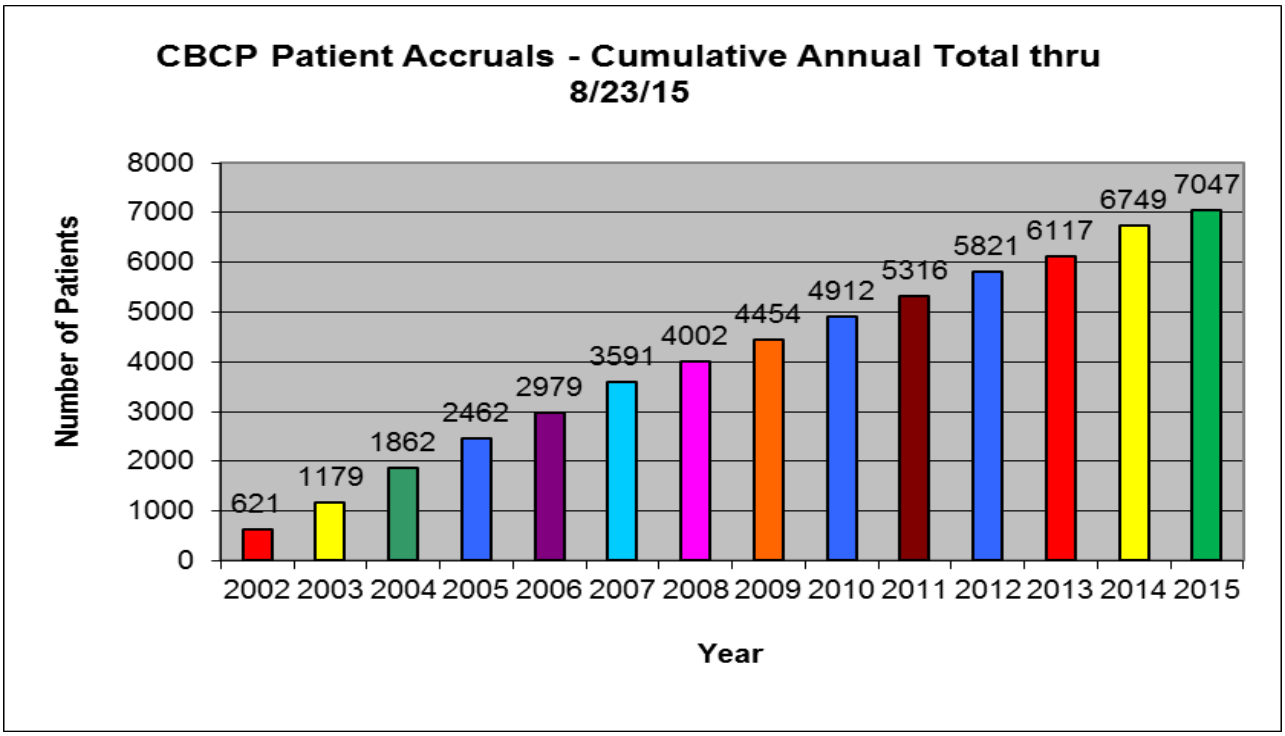


Figure BB-2. Cumulative patient accruals into CBCP protocols since 2002.

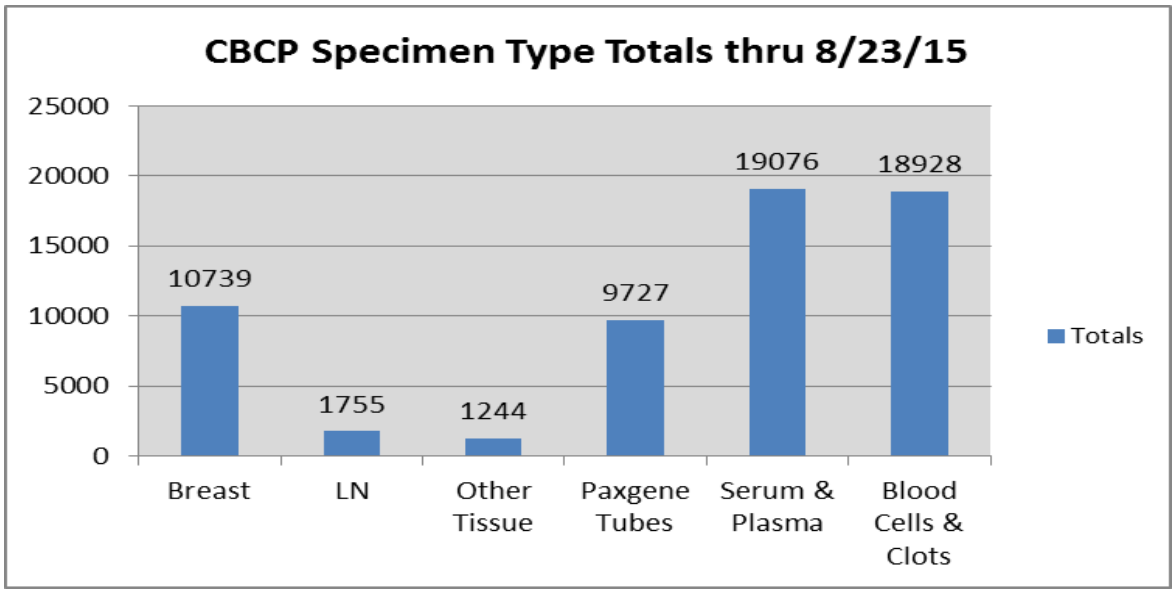


Figure BB-3. The numbers and types of biospecimens collected by the CBCP

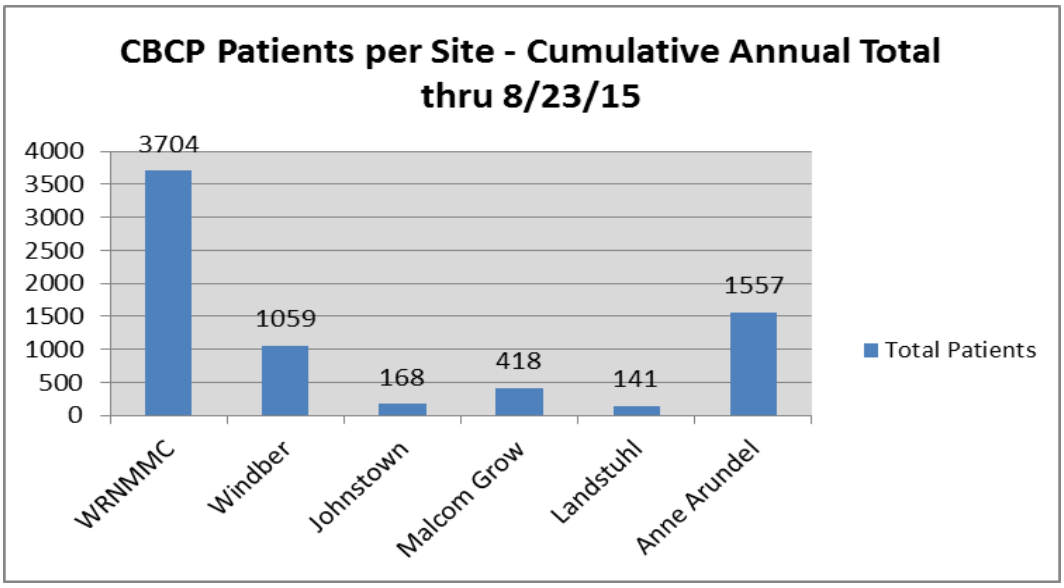


Figure BB-4. Numbers of patient recruited to CBCP protocols at various partner sites.

These specimens represent a broad spectrum of tissues, blood and blood products (**Figure BB-3**) that are not only a unique and valuable resource for the BC-TRC but are also the substrates for our translational research program. Along with the biospecimens that have been collected from CBCP participants, each consented patient also provides nearly 800 field of demographic, medical, life and family history data as well as complete pathology data on donated tissues. Patients have been recruited from a number of partnering clinical intake sites over the history of the CBCP (**Figure BB-4**). At the start of the BC-TRC the active partners are WRAMC, the Joyce Murtha Breast Care Center in Windber, PA, and the Anne Arundel Medical Center in Annapolis, MD.

III. Focused Research:

The ultimate goal of all BCTR-CoE research projects is to generate new knowledge that will benefit breast cancer patient treatment. The large volume of molecular data from BCTR-COE patients, integrated with the clinicopathologic data including the highly valuable treatment and outcome information, provides a gold data mining opportunity for BCTR-CoE scientists to generate new hypotheses for study and validate new experimental findings. This opportunity is even more enriched by the availability of large-scale high-quality datasets such as those from TCGA across multiple cancer types. Such raw data, combined with public annotation databases on genes, proteins, pathways, and human diseases, will enable derivation of new knowledge for breast cancer patient treatment.

There are two themes for BCTR-CoE research. Theme 1 focuses on breast cancer mechanistic studies of clinically important questions, and Theme 2 focuses on therapy-relevant molecular studies of breast cancers. For Theme 1 studies, one important topic is integrative profiling of breast cancers. The current 4 major breast cancer subtypes—termed “intrinsic subtypes”—were based on gene expression profiling. IHC-based subtyping using ER, PR, Ki67 and HER2 are available and are of clinical

significance, although such subtyping is sometimes referred to as surrogate for intrinsic subtyping. Information on a broader panel of proteins and their post-translational modifications as well as their subcellular location information is needed for a more comprehensive understanding of breast cancer stratification which is important for cancer treatment. Thus such studies are important not only for Theme 1 but also for Theme 2, for example, the identification of protein markers for endocrine resistance.

For Theme 1 studies, the BCTR-COE provides a good research environment on young breast cancer patients and African American patients. Young age at breast cancer diagnosis and being African American are considered risk factors for poor outcomes of breast cancer patients. BCTR-COE has enrolled a high percentage of AA patients, and there is also a good size of young breast cancer patients enrolled due to the demographics of the active-duty military population. Using these resources BCTR-COE scientists have conducted molecular studies, and have proposed additional molecular, epidemiologic, and comparative survival analysis using both BCTR-COE data and the data in the public domain.

The topic of tumor heterogeneity is not only important to the understanding of breast cancer development (Theme 1), but also of therapeutic significance (Theme 2). Tumor heterogeneity refers to the cellular heterogeneity of tumor development environment, where there are cancer cells, stromal cells, lymphocytes, etc., and the MCC has chosen "Inflammation, Infection, Immunity, and Stroma (I3S) as one of the focuses for research. Tumor heterogeneity also refers to the fact that one physical tumor could contain multiple lineages of tumors that are not necessarily of the same molecular subtype. When only one subtype was diagnosed and treated, the other subtypes could be left untreated which could lead to detrimental outcome of the patient.
Additional topics are proposed to be studied on mechanistic understanding of breast cancer development. These include genetic dispositions, exposure to environmental risks, access to healthcare and treatment disparities, and impact of certain life style factors as well as comorbidities.

For Theme 2 studies, profiling of human biospecimens alone is important but insufficient; biospecimens are no longer alive after excision from the human body, and in order to study the impact of drugs or the response to drugs of a mutated gene, a live model system is needed. BCTR-COE scientists has developed tissue culture systems for both 2D and 3D model systems of breast cancer cell lines, with a focus on the triple-negative subtype that are currently difficult to treat. Findings from such studies are validated or sometimes guided by bioinformatics analysis of the data on human tissues.

IV. Biomedical Informatics:

As one of the five pillars of the CBCP, Biomedical Informatics (BMIX) has developed a comprehensive informatics system supporting the activities in all of the other 4 pillars. Biomedical Informatics also provides support to other research projects and leads its own research, by working with scientists both within and outside of the WRI. In the recent years, the BCTR-CoE has been conducting or participating in several large-scale molecular studies, including the TCGA-BC, Massive Parallel Molecular Processing in collaboration with the Pacific North Western National Lab, a Komen Promise Grant for therapy relevant molecular stratification of breast cancers in collaboration with

Thomas Jefferson, etc. New initiatives are in development. The BCTR-CoE is now also addressing the collection of treatment and outcome data for invasive cancer patients enrolled in the study. These projects, combined with the research conducted by scientists at the WRI, has generated a large amount of molecular data as well as new types of clinical data. It is thus critical to expand our current informatics infrastructure to manage all these data, and more importantly, it is critical we expand our bioinformatics research capability to conduct integrative analysis to analyze these data, mine for new hypothesis for validation both computationally and experimentally, so as to make the best use of the data towards making important findings in understanding cancer development mechanisms, identifying cancer treatment drug targets, and develop physician decision support system to aid in cancer treatment.

Biomedical Informatics is now broadly defined as a multi-disciplinary subject for the management and utilization of biomedical information encompassing clinical informatics, public health informatics, and bioinformatics [2]. This definition is increasingly important as new concepts and technologies enter into medical practice and related basic research, and require new types of information management and data analysis that relies on sophisticated statistical and computational technologies. Figure DD.0 shows the major components in this definition of BMIX [3].

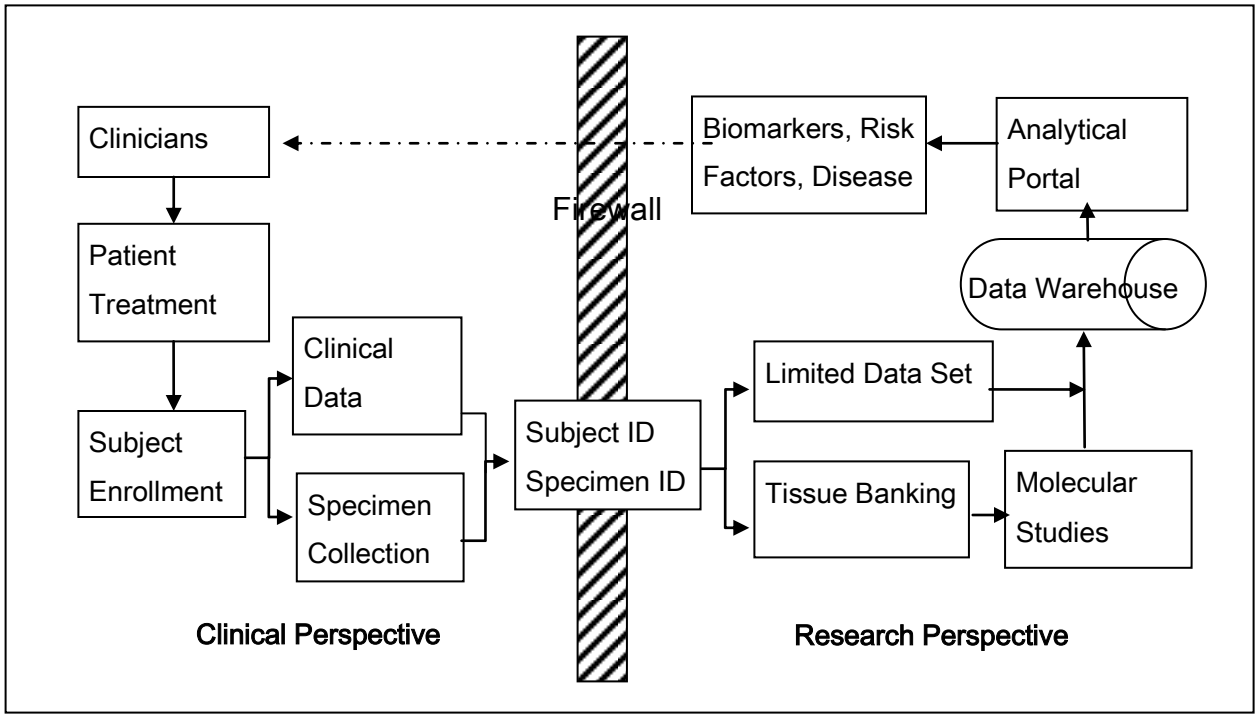


Figure DD.0. Major components of biomedical informatics. Clinically, patients receive treatment, subjects are enrolled in the study, and clinical data as well as specimens are collected. To protect the privacy of human subjects, de-identified subject IDs and specimen IDs are created and properly mapped before being transferred to the research side with the corresponding clinical data and the specimens. On the research side, clinical data are properly stored, tissues properly banked and genomic and proteomic studies conducted. All data are then warehoused, analyzed, and mined for biomarkers, risk factors, and disease models. Newly obtained knowledge is fed back to the clinic to aid in clinical decision-making.

From the data flow point of view, these BMIX components include 1) supporting data collection and generation across clinical, genomic, and proteomic platforms, 2) data tracking, 3) data centralization, 4) data analysis and mining, and 5) knowledge generation and presentation to research and clinical applications. We have been working towards developing a complete BMIX infrastructure for the BCTR-COE. The system we are developing was designed to be flexible to enable expansion to support translational research in other disease areas. In the following we will present the background, the current status, and the plan for each of these 5 components of BMIX.

V. Translational Clinical Care:

The objectives of the Clinical Care Pillar are to:

- Decrease the negative psychological impact on the patient of having an evaluation or treatment intervention for breast disease by utilizing objective measurement instruments to longitudinally assess the patient's psychological response to evaluation and intervention, and base modifications of these procedures on those results.
- Create and maintain an environment (medical, physical, psychological) conductive to the multiple needs of the patient undergoing breast disease evaluation / treatment.
- Recruit patients into the various BCTR protocols to obtain the clinical data and biospecimens needed to meet the BCTR's translational research goals.

This pillar of the BCTR is the foundation upon which all the success of and project rests. Without patients enrolled in our biospecimen repository protocols, there would be no translational research center of excellence. These patients come from the clinical care environment. Since its inception in 2000, the CBCP (now the BCTR-CoE) has had as a priority, the development and staffing of the core clinical centers at Walter Reed National Military Medical Center, the Joyce Murtha Breast Care Center in Windber, PA and at our newest site, the Pat and Lesly Sajack Breast Center at Anne Arundel Medical Center in Annapolis, Maryland. Under the direction of Lorraine Tafra, MD more than 500 newly diagnosed cases of breast cancer are seen at AAMC each year.

At each center the staff is dually trained as clinical/research providers, to seamlessly integrate the need for a strong research focus in the clinical center with the requirement to provide state-of-the-art clinical care to the patients.

The reputation of the BCTR is that of an exceptional translational research project with very possibly the world's most pristine collection of breast tissue. This has resulted in a number of well-respected medical centers expressing interest in joining us as research partners.

The care of our patients is provided by Physicians, Advance Practice Nurses (Nurse Practitioners) and certified Nurse Navigators with all personnel having as their prime job description, the research aspects of the BCTR.

Walter Reed National Military Medical Center in Bethesda, MD has a state of the art comprehensive breast care center with women's imaging co-located with the breast care center. The Breast Center has

a procedure room and recovery room enabling surgery within the center. The Breast Imaging Center has a designated Aurora Breast MRI machine. We anticipate seeing between 7,500 patients per year and diagnose approximately 250 new breast cancers per year. The Breast Care and Translational Research Center of Excellence received a 3 year full accreditation by the NAPBC (National Accreditation Program for Breast Centers) on September 11, 2012, making BCTRCOE accredited through September 2015. We are currently preparing for our upcoming accreditation site visit scheduled for September 10, 2015, with expectations to be accredited through 2018.

4. KEY RESEARCH ACCOMPLISHMENTS:

Breast Cancer Translational Research Center of Excellence (BCTR-COE)
Statement of Work

Task 1: Identify and counsel 100 patients annually at high risk for development of breast cancer, and employ risk reduction strategies.

Ongoing
Dr. Raymond Weiss, Medical Oncologist saw 310 patients in the Breast Care and Research Center at Walter Reed National Military Medical Center in Bethesda, MD and conducted 79 teleconferences with patients from 23 August 2014 – 24 August 2015.

Task 2: Accrue over 500 patients annually to the "core" BCTR-COE protocols through consenting patients in the main BCTR-COE clinical sites.

Ongoing
Total Patients consented from 23 August 2014 – 24 August 2015.
Total Patients Collected From
WRNMMC: 181
Windber: 65
AAMC: 156
****Research placed on hold at JMBCC for 9 months while a new PI was assigned, as a result numbers declined, which affected our annual totals.**

Task 3: Acquire through consented protocol acquisitions, over 5,000 specimens annually (neoplastic and non-neoplastic breast tissues and tumors, lymph nodes, metastatic deposits, blood and its components, bone marrow) on patients with all types of breast diseases and cancer.

Ongoing
Total Samples Collected from 23 August 2014 – 24 August 2015.
Total Samples Collected
Total Blood: 2244

Total Breast: 1003
Total LN: 60
Total Other: 150

****Research placed on hold at JMBCC for 9 months while a new PI was assigned, as a result numbers declined, which affected our annual totals.**

Task 4/5: Bank these biospecimens in the BCTR-COE Biorepository as the substrate for all molecular analyses carried out in BCTR-COE labs, as outlined in the BCTR-COE Core Protocols. Utilize this repository as the basis for intramural and extramural collaborations for secondary usage research.

Ongoing
Weekly reports are issued from Windber Research Institute. For week ending 8/23/2015, there were a total of 61,469 biospecimens banked from a total of 7,049 consented subjects.

Task 6: Perform whole genome DNA sequencing on DNA from 40 or more cases of breast cancer over the life of the project.

There was no new case analyzed.

Task 7: Develop and support a robust laboratory information management system to ensure proper tracking of data acquisition and a clinically relevant and laboratory research-linked prospective, longitudinal computerized data warehouse to support translational research and ultimately support physician decision making.

Ongoing

- *We continued development of the CLWS replacement. We finished system architecture, data dictionary, database design, and technology selection. Following the training on the new development model (Agile), we started to use it.*
- *We participated in the designing of the Case Report Form (CRF) and started implementing it in CLWS replacement. We finished the development of the Patient Registration, Protocol Assignment, and part of the CRF modules and released them to the users for initial testing.*
- *Working with the Tissue Bank group we learned that a number of reports that are manually generated weekly and the Sample Shipment Receiving and Reconciliation process are not supported by the current CLWS. We are considering including these functions as part of the CLWS replacement.*
- *We investigated a SOAP module for FreezerWorks that would allow us to automatically synchronize data between the CLWS replacement system and FreezerWorks.*

Task 8: Develop an analytical system for integrative data analysis and mining, and develop a breast knowledgebase to support clinical and research activities in BCTR-COE.

Ongoing

- *We started preparation of legacy data conversion from the old CLWS and Data Warehouse systems to the CLWS replacement. Using the Data Dictionary, we started to develop an Analytical Workflow that will implement conversion rules and will integrate data from multiple data sources into the CLWS replacement. We finished clinical data conversion and continue working on pathology, treatment and outcome data.*
- *We continued loading data to the Data Warehouse and developed a process of preparing summary reports for participating clinical sites and sent them to corresponding parties. We finished archiving experimental results files and identified most of the projects for microarray on blood and tissue.*
- *In collaboration with the tissue bank we developed the WRI-CBCP Project and Sample Request and Approval Form and the WRI-CBCP Publication Clearance form that will help standardize the Sample Request process including using standardized project names and specifying sample types, and will improve the mechanism for flagging available samples for research and tracking the experimental results derived from those samples.*
- *We integrated outcome and biomarker data collected through different programs to data warehouse. We finished automation the data warehouse loading and tested it with new load. We finished collecting experimental results files and identified most of the projects for microarray on blood and tissue. We also identified a list of possible software that can be used for developing a knowledgebase.*

Task 9: Conduct quantitative analysis of therapy relevant proteins by immunohistochemistry within subclasses of breast cancer to provide better patient selection into clinical trials for targeted and combination therapies.

- *A proof of principle of the analysis for 27 markers have been performed for over 200 cases, connecting the information on ER, PR, and HER2 to other markers for increased understanding of molecular connectivity in breast cancer tumors.*
- *Analysis of markers CD163 and phosphohistoneH3 has been performed with has been shown to be associated with patient survival and patient or tumor characteristics. The results have been submitted to SABCS 2015. A manuscript is in preparation.*
- *Additional markers and sets of markers have been analysis, with observations confirmed reported results. Some analyses, such as the HER family receptors, more cases would be needed to derive a robust result.*
- *A preliminary Bayesian analysis of the whole complete dataset has also been performed with observations confirming known results. More cases will be needed in order to make robust new findings.*

Task 10: Study molecular differences between breast tumors from African American and Caucasian women as the identification of such differences will allow for the development of more effective therapies that will improve outcomes in African American women with breast cancer.

- *Project complete. Tumors are not molecularly different and thus disparities are not attributable to molecular factors. Publication reporting effects of population stratification submitted for publication.*

Task 11: Using state-of-the-art 3D cell culture techniques and modern approaches to the study of cancer cell biology, study the mechanisms of cell invasion, migration and ultimately metastasis in breast cancer cell lines.

Ongoing, several abstracts and publications have been presented on this topic, see below:

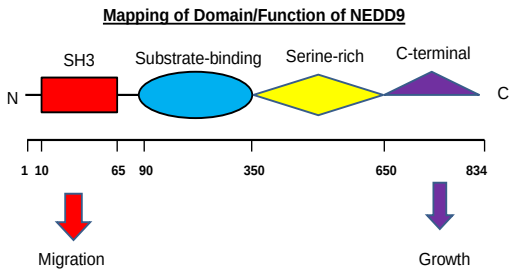
Aim 1. CSPG4-NEDD9 interaction promotes triple-negative breast cancer progression and metastasis.

There are lines of evidence demonstrating that NEDD9 (Cas-L, HEF-1) plays a key role in the development, progression, and metastasis of breast cancer cells. We previously reported that NEDD9 plays a critical role for promoting migration and growth of MDA-MB-231. In order to further characterize the mechanisms of NEDD9-mediated cancer migration and growth, stable cells overexpressing NEDD9 were generated using HCC38 as a parental cell line which expresses low level of endogenous NEDD9. Microarray studies demonstrated that core proteins of CD44 and Serglycin were markedly upregulated in HCC38(NEDD9) cells compared to HCC38(Vector) cells, while those of Syndecan-1, Syndecan-2, and Versican were downregulated in HCC38(NEDD9). Importantly, enzymes generating chondroitin sulfate glycosaminoglycans (CS) such as CHST11, CHST15, and CSGALNACT1 were upregulated in HCC38(NEDD9) compared to HCC38(Vector). Immunofluorescence studies using specific antibody, GD3G7, confirmed the enhanced expression of CS-E subunit in HCC38(NEDD9). Immunoprecipitation and western blotting analysis demonstrated that CS-E was attached to CD44 core protein. We demonstrated that removing CS by chondroitinase ABC significantly inhibited anchorage-independent colony formation of HCC38(NEDD9) in methylcellulose. Importantly, the fact that GD3G7 significantly inhibited colony formation of HCC38(NEDD9) cells suggest that CS-E subunit plays a key role in this process. Furthermore, treatment of HCC38(NEDD9) cells with chondroitinase ABC or GD3G7 significantly inhibited mammosphere formation. Exogenous addition of CS-E enhanced colony formation and mammosphere formation of HCC38 parental and HCC38(Vector) cells. These results suggest that NEDD9 regulates the synthesis and expression of tumor associated glycocalyx structures including CS-E, which plays a key role in promoting and regulating breast cancer progression and metastasis and possibly stem cell phenotypes.

Publication: Role for chondroitin sulfate glycosaminoglycan in NEDD9-mediated breast cancer cell growth. Joji Iida, Jesse Dorchak, Rebecca Clancy, Juliana Slavik, Rachel Ellsworth, Yasuhiro Katagiri, Elena N. Pugacheva, Toin H van Kuppevelt, Richard J. Mural, Mary Lou Cutler and Craig D. Shriver. Experimental Cell Research, 330(2):358-70, 2015

Presentation: Tumor-associated glycans as key molecules to promote triple-negative breast cancer cells. Joji Iida, Jesse Dorchak, Rebecca Clancy, Juliana Slavik, Mary Lou Cutler, Craig D. Shriver. San Antonio Breast Cancer Symposium, 2015, San Antonio, TX.

During the period, we further characterized mechanisms of NEDD9-driven malignant phenotypes of triple-negative breast cancer. As summarized in Figure 1, we demonstrated that the SH3 domain promotes migration and the C-terminal domain promotes growth. Given the enhanced migration and growth are hallmarks of malignant cancer cells, our results suggest that NEDD9 promotes triple-negative breast cancer invasion and metastasis through distinct non-overlapped domains.



In order to identify partner proteins to the C-terminal domain, we performed yeast-two hybrid (Y2H) assays using the domain as bait

to screen commercial available human cDNA library. As a result, we obtained more than 300 candidate proteins that associate with the C-terminal domain of NEDD9. We are currently further eliminating false-positives. The next step of this project is to 1) characterize the interaction in triple-negative breast cancer cells using immunoprecipitation/western blotting analyses and 2) evaluate the interaction for promoting breast cancer growth.

We are currently identifying key residue(s) that promote triple-negative breast cancer growth by introducing various point mutations in the domain. Once we identify the key residue(s), we will identify partner proteins by either Y2H or pull-down studies using Mass Spectrometry Analysis.

Aim 2. Development of DNA aptamers against CD44 that inhibit breast cancer invasion and metastasis.

During the previous period, we developed novel and unique DNA aptamers that specifically bound to exon v10 of CD44 using Systematic Evolution of Ligands by Exponential Enrichment (SELEX) systems. These DNA aptamers inhibited TN breast cancer migration toward type I collagen, suggesting that this domain plays a role in promoting breast cancer metastasis by enhancing migration toward tissue architecture in vivo. We further characterize CD44v10-mediated migration and identified EphA2 was a binding partner to this domain. We demonstrated that the DNA aptamers that inhibited migration also prevented the association of EphA2 with CD44v10. These results suggest that CD44 forms a molecular complex with EphA2 on the breast cancer cell surface and this complex plays a key role in enhancing breast cancer migration. These results provide insight not only for characterizing mechanisms of breast cancer migration but also for developing target-specific therapy for breast cancers and possibly other cancer types expressing CD44 exon v10.

In this period, we are characterizing CD44-EphA2 association for promoting malignant phenotypes of breast cancer cells using T47D and SKBr3 as model systems, since these cells express under detectable level of endogenous EphA2 and CD44. We demonstrated that ^{594}Y, ^{588}Y, ^{772}Y, and ^{897}S were phosphorylated by western blotting analyses in the both of cell lines. The over expression of EphA2 alone did not affect migration of these cells to type I collagen. We are currently evaluating CD44 for regulating phosphorylation of these residues and migratory phenotypes.

Aim 3. Identification of drug-targets for triple-negative breast cancer.

SOX10 is a transcription factor and plays a role in neural crest development. Recent studies demonstrated that SOX10 is a driver of melanoma progression as developing from melanocytes which is neural crest cell origin. In breast cancer tissues, SOX10 expression is a potential diagnosis marker especially in basal-like breast carcinoma. Thus, these results suggest that characterization of the mechanisms of SOX10-driven malignant phenotypes of cancer cells would provide significant insights for novel diagnostics and therapeutic tools.

In order to evaluate biological functions of SOX10, we knocked out its expression by shRNA in HCC38, which express high level of SOX10. When the expression of SOX10 was inhibited, cells died. Accordingly, overexpression of SOX10 in HCC1806 (expressing low endogenous SOX10) stimulated cell growth. These results suggest that SOX10 plays a key role in promoting proliferation of breast cancer cells.

Phosphopeptide analyses of total cell lysates from SOX10^{+}breast cancer cells suggests that Ser^{45} and Ser^{24} could be phosphorylated in SOX10. In order to characterize the phosphorylation of Ser^{45}, we overexpressed mutant of Ser^{45}(A) and Ser^{24}(A)of SOX10 in HCC1806 cells and tested their growth ability. Our results suggest that mutation of both Serine residues failed to stimulate growth, suggesting that the modifications of these two residues are important for promoting growth.

In order to identify proteins that associate with SOX10, we immunoprecipitated SOX10 from cell lysates of HCC38 and subjected to mass spectrometry studies. We found zyxin as one of the SOX10-associated proteins. By co-immunoprecipitation and western blotting analysis, we demonstrated that ^{45}S but not ^{24}S of SOX10 plays a key role in associating with zyxin. Previous studies suggest that zyxin plays a key role in promoting cancer cell migration. Given that enhanced migration is one of the key features of malignant cancer cell phenotypes, our results suggest a novel role of transcription factor, SOX10, acts as a cytoskeletal protein for promoting migration. We are currently characterizing SOX10-zyxin interaction on molecular basis.

Aim4. Development of novel Ruthenium (Ru)-compounds as anti-cancer reagents.

Previous studies suggest that transition metal complexes, such as cisplatin, are efficacious for treating various cancer types, including ovarian, lung, and breast. In order to further evaluate ruthenium (Ru) complexes as potential anti-cancer agents, we synthesized and evaluated ruthenium (Ru) –arene complexes. Two complexes with the general formula [Ru (η^6-*p*-cym) (N-N) Cl] $^{+}$ were tested for their abilities to inhibit cancer cells. The complex with *o*-phenylenediamine (*o*-PDA) significantly inhibited growth of breast (MDA-MB-231, MCF-7, SKBR-3, and SUM149), lymphoma (Raji), melanoma (Bowes), and osteosarcoma (HT1080); however, complex with o-benzoquinonediimine (*o*-BQDI) was

ineffective. In contrast, *o*-PDA failed to inhibit growth of human breast epithelial cells, MCF-10A cells. Treatment of MDA-MBA-231 cells with o-PDA resulted in a significant reduction of productions of PDGF-AA, GM-CSF, and VEGF proteins at the transcriptional levels. Finally, we demonstrated that o-PDA synergistically inhibited MDA-MB-231 cell growth with cyclophosphamide but not doxorubicin and paclitaxel. These results suggest that Ru-arene complexes are promising anti-cancer drugs that inhibit progression and metastasis by blocking multiple processes for patients with various forms of cancer.

Publication: Inhibition of cancer cell growth by ruthenium complexes
Joji Iida, Elisabeth, T. Bell-Loncella, Marc, L. Purazo, Yifeng Lu, Jesse Dorchak, Rebecca Clancy, Julie Slavick, Mary Lou Cutler, and Craig D. Shriver Cancer Research, *Submitted*

Presentation: Structure-Activity Relationship of Ruthenium (Ru) Complexes to Inhibit Breast Cancer Growth and Metastasis. Joji Iida, Marc L. Purazo, Yifeng Lu, Elisabeth, T. Bell-Loncella, Craig D. Shriver San Antonio Breast Cancer Symposium, 2014, San Antonio, TX

Task 12: Use our unique collection of breast cancer biospecimens to characterize microRNA (miRNA) expression in breast cancer progression and metastasis.

- *This project is on hold.*

Task 13: Identify protein signatures associated with the development and progression of pre-malignant breast disease to improve our understanding of the biologic processes involved in early breast disease development and progression and to drive the development of personalized therapeutics for breast disease.

- *This project is currently inactive.*

Task 14: Identify genetic changes in low- and high-grade breast tumors to improve our understanding of the evolutionary process of breast cancer and to identify a protein signature that can discriminate low- from high-grade breast tumors, allowing for more accurate diagnosis and risk assessment.

- *Data generation completed. Manuscript combining gene expression and protein results in preparation.*

Task 15: Use our unique collection of breast cancer biospecimens to characterize molecular signatures that can differentiate primary breast tumors with and without metastatic potential, as well as between primary tumors and subsequent metastases.

- *Project completed. Results presented at Society of Surgical Oncology meeting March 2015 and published as* CD Shriver, MT Hueman, **RE Ellsworth**. Molecular signatures of lymph node status by breast cancer intrinsic subtype. J Exp Clin Cancer Res, 33: 782, 2014

Task 16: Improve our understanding of the molecular changes associated with HER2 amplification and over-expression to allow for more precise diagnosis of HER2+ patients and development of customized treatment options in patients with HER2+ breast cancer.

Objective 1 Evaluate differences in the molecular profiles of patients with increased HER2 expression.
Ongoing.

- *Preliminary data analysis showed a difference in gene expression profiles between patients with increased HER2 expression due to HER2 gene amplification (n=18) vs. chromosome 17 polysomy (n=14). To increase sample size, additional cases with HER2 copy number alterations have been identified and will be evaluated for gene and protein expression differences. We are also optimizing protocols to utilize RNA from archived formalin-fixed paraffin-embedded specimens for pathway-focused gene expression analysis as well as for the validation of any gene expression differences that are found in the comparison of frozen tissue specimens.*

Task 17: Study the role of matrix metalloproteinases in breast cancer with the goal of developing diagnostic and prognostic marker of breast cancer based on expression of MMPs and polymorphisms in MMPs.

- *Complete with 4 publications.*

Task 18: Identify molecular alterations in the breast tumor microenvironment that contribute to tumorigenesis and which may lead to improved methods of breast cancer prevention and treatment.

- *Adipose pilot study complete with publication in 2014. Complete.*

Task 19: Use our unique collection of breast cancer biospecimens to study angiogenesis and lymphogenesis in different grades of DCIS and IDC.

- *This project is on hold.*

Task 20: Incorporate the rapidly growing public genomic and proteomic datasets related to breast cancer into our data warehouse to be able to mine the combined data sets for the generation of new hypotheses regarding breast cancer development, progression and treatment.
Ongoing, outgrowth of project with NCI/NHGRI TCGA project. *Nothing new to report.*

- *Subaim 1. Generate a tissue-experiment inventory for TCGA-BC BCTR-CoE cases. Complete.*
- *Subaim 2. Integrate gene expression microarray data for both Level 1 and Level 3 data. Complete for Level 3 and decided that Level 1 is not as important.*
- *Subaim 3. Develop applications to use the integrated gene expression data. Complete at the query level and decided that more advanced applications will not be cost-efficient,as our resources are limited.*

- *Subaim 4. Integrate Level 3 DNA Sequencing data, and make results available to scientists, using similar approaches. Complete, and same logic for applications study.*
- *Subaim 5. Integrate SNP data and make the results available to scientists using similar approaches. This type of data is not immediately needed so this subaim is on hold.*

Task 21: Compare biomarker expression in core biopsy and surgically resected tumors. This analysis is exploring whether the expression of biomarkers as measured by IHC are higher on core biopsies than surgically resected tumors, and whether such differences may impact the sub-typing of the breast cancer from the patient.

Comparing biomarker expression in core biopsy and surgically resected tumors.

- *Complete with a poster presented at SABCS 2013.*

Task 22: Use the biomarkers of ER, PR, HER2, and Ki67 by IHC to classify luminal invasive breast cancers into LA, LB1 (HER2-), and LB2 (HER2+).

HER2+ and HER2- luminal B subtypes of invasive breast cancers.
Ongoing.

- *Complete for the performance period with a poster presented at the SABCS 2013. Additional analysis will be performed as more outcome data are available towards a publication.*

Task 23: Use bio-specimen research activities to evaluate the effect of a variety of pre-analytical variables on samples collected for tissue banking.

Maintaining efficient Quality Management Systems (QMS) to provide quality tissue for research. Performing Biospecimen Research activities so that data obtained will be utilized to design data driven protocols and procedures. These activities will help the biobank maintain the integrity of its biospecimen and thus provide a biorepository environment that meets the industry's standards.

College of American Pathology (CAP) Accreditation
The biobank received CAP accreditation in May, 2015 after a successful inspection on April 7th, 2015. The accreditation covers the services below

- ✓ Biorepository General
- ✓ General Specimen Processing
- ✓ Nucleic Acid Extraction
- ✓ Specimen Collection/Procurement
- ✓ Specimen Distribution
- ✓ Specimen Informatics
- ✓ Specimen Storage

Quality: Maintaining tissue integrity is essential to obtaining quality nucleic acids from these tissue specimens. Nucleic acids are one of the main byproducts of the tissue that are utilized for downstream

experiments. The quality of the specimens will determine the ability to effectively translate these experimental results from the laboratory to the clinic. Periodically we monitor biospecimen quality through the analysis of nucleic acid quality parameters. Data analyzed shows that the biospecimens collected have continued to provide nucleic acids of good quality for research as shown in Table 1 below.

Table 1-RNA quality parameters between 2008 and 2014

	Number of Samples per year						
RIN #	2008	2009	2010	2011	2012	2013	2014
≤ 5.0	0 (0%)	5 (4%)	6 (4%)	2 (1%)	0 (0%)	2 (4%)	0 (0%)
5.1-5.9	0 (0%)	4 (4%)	7 (5%)	11 (5%)	0 (0%)	2 (4%	2 (4%)
6.0-6.9	7 (19%)	26 (22%)	25 (17%)	36 (17%)	3 (7%)	7 (14%)	10 (21%)
7.0-7.9	21 (57%)	41 (35%)	67 (47%)	76 (35%)	22 (51%)	20 (41%)	23 (47%)
≥ 8.0	7 (19%)	17 (15%)	21 (15%)	19 (9%)	10 (23%)	10 (21%)	9 (18%)
NA	2 (5%)	23 (20%)	17 (12%)	72 (33%)	8 (19%)	8 (16%)	5 (10%)
Total # samples	37	116	143	216	43	49	49

The data shows that for 653 OCT blocks utilized for laser microdissection, 94% of the samples provided RNA of acceptable quality based on RNA integrity Numbers (RIN), Figure 1.

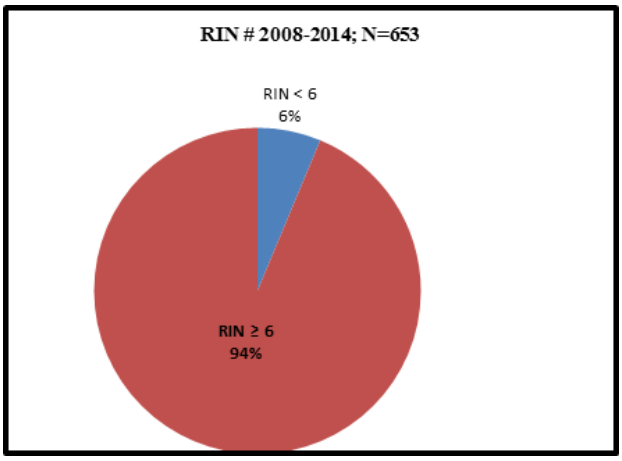


Figure 2- RNA integrity for laser micro dissected samples between 2008 and 2014.

We also observed that DNA quality was maintain in the specimens collected and processed as shown in Table 2 below. The ratio of absorbance at 260nm and 280nm is used to assess DNA purity (and RNA). A ration of ~ 1.8 is generally accepted as “pure”. For DNA

Table 2: Data on sample DNA purity.

Sample #	Purity (260/280 ratio)	Percentage
733	≥1.8	97.7
14	1.5 - < 1.8	1.9
3	< 1.5	0.4

Proficiency Testing

The biobank participated in a proficiency testing program conducted by the Integrated Biobank of Luxembourg (IBBL). This program is also endorsed by the International Society for Biological and Environmental Repositories (ISBER), the leading global organization for biobanks. The biobnak tested successfully in the following proficiency areas.

- DNA Extraction from Whole Blood
- DNA Quantification and Purity scheme
- DNA Extraction from FFPE Cells
- RNA Integrity Scheme
- Tissue Histology Scheme

In our continued efforts to improve quality, consistency and reliability in our operations, we performed a number of quality assurance activities which included audits. A listing of these activities is shown on Table 3.

Table 3: Quality assurance initiatives performed during the period 8/2014 to 3/2015.

DATE	Quality Assurance Activity
8/1/2014	Humidity & Temperature Validation Study - Biorepository
9/12/2014	Comprehensive WTR CORE Inventory Audit
10/16/2014	CBCP & CCBB Analysis of Errors & Corrections
11/7/2014	Customer Satisfaction Results Analysis
1/7/2015	Audit of signed-out tissue samples in -80 freezer
1/21/2015	CBCP-Tissue Quality (RIN) of LMD Samples
2/25/2015	AAMC Blood Collect/Receive/Freeze Times
2/25/2015	WRNMMC Blood Collect/Receive/Freeze Times
2/25/2015	JMBCC Blood Collect/Receive/Freeze Times
3/10/2015	LN Usage study

Documentation:

We have put in place systems to enable us track as much information as possible from the moment the

specimen is obtained from the donor through all the processes leading to and including its storage. Also, quality management systems are in place to monitor and evaluate our activities through data generated from audits and other documentation processes that are in place. Standard Operating Procedures (SOP) continue to be designed to allow efficient operations and processes at all levels of the biobank's daily activities. Currently, we have 96 active SOPs and more than 76 Logs or Forms for documentation. These all help us achieve the required standards and Best Practices of the industry. A summary of the SOPs generated to date is shown in Table 4 below.

Table 4: SOP- 2003 to August 2015

Year	Total SOP developed (cumulative)
2003	5
2006	31
2008	33
2010	58
2012	62
2014	88
2015	96

Education and Training- We continue to explore avenues for improving our knowledge and keeping abreast with new developments in the industry. The following activities are reported for staff;

- Caroline Larson/Stella Somiari- earned CEU credits in *Quality Assurance (*Workforce Dev. & Continuing Ed/Southern Alleghenies Workforce*).* 8 CEU's earned per participant
- Stella Somiari, PhD received a Certificate in Audit (CQA) Fundamentals1
- Brenda Deyarmin- certified in *Immunohistochemistry* by American Society of Clinical Pathology
- Caroline Larson/Amber Greenawalt- FreezerWorks Training-*User Education Conference*

Below (Table5) is a list of webinars which employees participated in.

Table 5: Webinars participated in during period of report

Topic	Speaker	Objective
6-3-2015 **Advances in addiction research: applying genetic biomarkers to personalized treatment.**	**Andrew Brook, PhD** **James Baurley, PhD**	**Sample management, importance of functional quality control of biomaterials, best practices for discovery and translational research, advantages of fully automated genotyping platforms,**
5/20 -5/21-2015 **The GTEx symposium: All things considered-biospecimens, "omics, data and ethical issues**	**Multiple speakers** **NCI/NHGRI/NIMH, NIH Common Fund**	**Highlight of the scientific goals of the GTEx program, progressing in collecting and analyzing biospecimens from over 25 organ sites from 900 post mortem donors.**
4-15-2015 **CAP: Maximize your existing QMS to deliver great lab value**	**Caroline Maurer (Director CAP 1589 Program)** **William Castellani, MD**	**Review of key elements of a QMS that fulfils CAP requirements and ISO 15189**
3-11-2015 **Commercial biorepositories-BioStorage Technologies**	**Video**	**Understand and familiarize with the operations of large commercial biorepositories**
1-20-15 **Biobank Financial Stability**	**Jim Vaught, PhD (Current ISBER President)** **Marshall Schreeder (CEO, ConversantBio)**	**Informational webinar on the ConversantBio biobank granting opprortunity**
12-2-2014 **Laser Microdissection Perfection**	**Dr. Nicola Funel** **Dr. Christian Lobinger**	**Laser microdissection techniques for success.**
8-4-2014 **Optimizing samples for future use: Innovative technology to improve the functional quality control of DNA samples**	**Andrew Brooks, PhD (COO RUCDR Infinite Biologics/Rutgers University** **Tatiana Foroud, PhD (Professor Medical & molecular Genetics/Director Hereditary Genomics Indiana University School of medicine**	**Learn about SNPtrace™ technology for sample quality/integrity assessment. Best practices role in research applications leading to translational medicine.**

Biospecimen Research: Experiments are ongoing in the area of biospecimen science research to support our SOPs and move them to evidence-based SOP. Current research continues in the areas of new collection methods and determining nucleic acid quality of such new methods. Below is a summary of the activities performed during this reporting period.

- Laboratory work for the experiment focusing on the potential of biopsy imprints as a source nucleic acid has been completed and data is being analyzed
- The experiment to determine the effect of different processing methods on the DNA quality from biopsy specimens has been completed and data is being analyzed.

Task 24: Evaluation of molecular and epidemiological data associated with outcome disparities in African American women with breast cancer

- *Objective 1: Complete.*
- *Objective 2: Outcome data is required from WRNMMC which has not yet been provided.*
- *Objective 3: Complete with negative results.*

Task 25: Evaluation of molecular and epidemiological data associated with outcome disparities in Young Women with breast cancer.

- *Objective 1: Demographic and path analysis was completed and presented at AACR. Treatment data not yet provided by WRNMMC.*
- *Objective 2: Completed. Results will be prepared in manuscript form in 2015.*
- *Objective 3: Completed. Results will be prepared in manuscript form in 2015.*

Task 26: Indentify blood-based signatures of breast disease that help in the generation of gene expression data from breast cancer patients with and without metastasis from blood, generation of gene expression data from control patients without breast disease and generation of protein expression data from serum from patients with and without metastatic disease using Discovery Map arrays.

Complete.

- *Objective 1: Completed. Produced negative results.*
- *Objective 2: Completed. Negative results were yielded.*
- *Objective 3: generation of protein expression data from serum from patients with and without metastatic disease using DiscoveryMap arrays.*

  This project has not begun. Currently, no agreement exists between WRNMMC and Myriad. We do hope to carry out this project however it will have to be paid for with CBCP supply funds rather than Myriad offering to run the samples for free.

Task 27: Analyze effect of a diagnosis of invasive breast cancer on lifestyle choices.

- *Objective 1. Data from lifestyle factors including fat intake, alcohol and tobacco use, exercise, BSE, HRT use and BMI will be collected from patients diagnosed with invasive breast cancer or benign breast disease who have filled out core questionnaires from baseline and follow-up visits The data collation is complete. Abstract/manuscript is in preparation.*
- *Objective 2. Statistical analysis will be performed to determine whether these factors improve in the invasive group and if they improve more significantly compared to the benign group. Data analysis completed. Corresponding abstract and manuscript to be completed by year's end.*
- *Abstract submitted to SABCS and corresponding manuscript has been submitted.*

Task 28: Assess the abundance and distribution of polychlorinated biphenyls (PCBs) in breast tissue.

The objective of this project is to assess the abundance and distribution of PCB congeners in human breast tissue through a comprehensive survey of mastectomy specimens from the Clinical Breast Care Project. Breast tissues have been collected from 302 quadrants from 62 patients with pathological diagnoses ranging from disease free prophylactic mastectomy samples to metastatic breast cancer. Analysis of 98 PCB congeners in these tissues has been conducted by pressurized liquid extraction followed by high resolution capillary gas chromatography. In collaboration with Paul J. Kostyniak, Toxicology Research Center, State University of New York at Buffalo.

- Manuscript published **RE Ellsworth**, K Mamula, B Deyarmin, PJ Kostyniak, D Gillard, CD Shriver, DL Ellsworth. Abundance and distribution of polychlorinated biphenyls (PCBs) in breast tissue. Environ Res 138: 291-297, 2015

Task 29: Examine genomic heterogeneity in primary breast carcinomas and among sentinel lymph node metastases: Implications for clinical management of breast cancer patients.

- *Abstract presented at SSO 2015. Manuscript has been published in Cancer Growth and Metastasis.*

5. CONCLUSIONS:

The annual goal of identifying and counseling 100 patients annually at high risk for development of breast cancer while employing risk reduction strategies was achieved.

BCTR-CoE successfully accrued and consented patients to our protocols, which continued to increase the specimen total in our biprepository. As of 23 August 2015 BCTR-CoE had banked more than 61K biospecimens in the BCTR-COE Biorepository, which are then used as the basis for intramural and extramural collaborations for secondary usage research.

During the year BCTR performed focused research on the biospecimens and clinical data collected under the BCTR-COE Core protocols, which resulted in multiple publications, abstracts and presentations by CBCP staff at peer-reviewed national meetings. National meetings included the San Antonio Breast Cancer Symposium, Society of Surgical Oncology Annual Cancer Symposium and the American Association for Cancer Research, Annual Meeting. See publications, abstracts and presentations on page 27.

A comparative survival analysis of patients with invasive breast cancer treated by a U.S. military treatment facility was conducted comparing BCTR-CoE's outcomes with with national database results (SEER). The summary of findings revealed that breast cancer patients from the Clinical Breast Care Project/BCTR-CoE at the Walter Reed National Military Medical Center showed a statistically significant advantage in disease-specific survival, overall survival, and 5-year survival rates over matched patients from the Surveillance, Epidemiology, and End Results program. Tumor characteristics explained only one-fourth to one-third of the 5-year survival rate differences at the whole cohort level.

The BCTR-CoE Biorepository underwent a thorough inspection on 4/7/2015 and successfully received its College of American Pathologists (CAP) Accreditation.

The Breast Care Center/BCTR-CoE recently underwent a thorough National Accreditation Programs of Breast Centers (NAPBC) inspection on 9/10/2015 and received an outstanding summary from the Surveyor where she described our Center as being excellent, we're meeting/exceeding all standards and there are "no discrepancies" with our program. It's expected that our Breast Care Center/BCTR-CoE will again receive the highest accreditation cycle of 3 years from the NAPBC in the coming months.

The BCTR-CoE at Walter Reed National Military Medical Center held its one day offsite meeting on 24 July 2014 at the Uniformed Services University of the Health Sciences. There were multiple presentations at the offsite meeting, which covered all areas of the CBCP, see attached Agenda (Attachment 3).

The John P. Murtha Cancer Center hosted its Second Annual Cancer Research Seminar on Monday 22 June 2015 from 8am-4pm at WRNMMC. There was a presentation given by one of the CBCP scientist on "NEDD9 as a potential therapeutic target for triple-negative breast cancer patients".

6. PUBLICATIONS, ABSTRACTS AND PRESENTATIONS:

CBCP Publications 24 AUG 2014 – 23 AUG 2015

Schinkel JK, Zahm SH, Jatoi I, McGlynn KA, Gallagher C, Schairer C, Shriver CD, Zhu K. "Racial/ethnic differences in breast cancer survival by inflammatory status and hormonal receptor status: an analysis of the surveillance, epidemiology and end results data."
Cancer Causes and Control. 2014 Aug; 25(8):959-68.

Cammarata FJ, Kvecher L, Rui H, Kovatich AJ, Campbell LF, Hooke JA, Joseph NP, Shriver CD, Mural RJ, Hu H. "A data repository system for translational research."
American Medical Informatics Association (AMIA) Annual Symposium, Nov 15-19, 2014, Washington, DC.

Joseph N, Kvecher L, Deyarmin B, Sturz L, Cammarata F, Larson C, Shriver CD, Mural RJ, Hu H. "Tissue-experiment inventory: a system to enable cataloguing of experimental results in association with tissue and participant information." American Medical Informatics Association (AMIA) Annual Symposium, Nov 15-19, 2014, Washington, DC.

Henning J, Oakes N, Valente AL, Deyarmin B, Meyer J, Hueman MT, Shriver CD, Ellsworth RE. "Evaluation of the role of EBV in breast cancer." Annual CTRC-AARC San Antonio Breast Cancer Symposium, Dec 9-13, 2014, San Antonio, TX.

Iida J, Purazo ML, Li Y, Bell-Loncella ET, Shriver CD. "Structure-activity relationship of ruthenium (Ru) complexes to inhibit breast cancer growth and metastasis." Annual CTRC-AARC San Antonio Breast Cancer Symposium, Dec 9-13, 2014, San Antonio, TX.

Ru Y, Lin J, Campbell JL, Zhu K, Kovatich AJ, Hooke JA, Kvecher L, Deyarmin B, Kovatich AW, Cammarata F, Rui H, Mural RJ, Shriver CD, Hu H. "Survival comparative analysis of patients with invasive breast cancer treated by a military medical center and matched patients of the U.S. general population." Annual CTRC-AARC San Antonio Breast Cancer Symposium, Dec 9-13, 2014, San Antonio, TX.

Ru Y, Mural R, Steeg PS, Rui H, Shriver CD, Hu H. "Age independently predicts worse disease-specific survival in patients diagnosed with invasive breast cancers at 50 years of age or older but not

younger." Annual CTRC-AARC San Antonio Breast Cancer Symposium, Dec 9-13, 2014, San Antonio, TX.

Shriver CD, Hueman MT, Ellsworth RE. "Molecular signatures of lymph node status by intrinsic subtype: gene expression analysis of primary breast tumors from patients with and without metastatic lymph nodes." Journal of Experimental Clinical Cancer Research. 2014 Dec 31;33(1):782.

Ellsworth RE, Valente AL, Hueman MT, Shriver CD. "Pathological and molecular effects of lack of PR expression in ER positive breast tumors." Cancer Research. 1 May 2015 75; P3-04-12. Annual CTRC-AACR San Antonio Breast Cancer Symposium; Dec 9-13, 2014, San Antonio, TX.

Yarina WC, Field LA, Deyarmin B, van Laar R, Hooke JA, Shriver CD, Ellsworth RE. "Molecular characterization of breast tumor-associated adipose." Cancer Research. 1 Jan 2015 75; B49.

Iida J, Dorchak J, Clancy R, Slavik J, Ellsworth R, Katagiri Y, Pugacheva EN, van Kuppevelt TH, Mural RJ, Cutler ML, Shriver CD. "Role for chondroitin sulfate glycosaminoglycan in NEDD9-mediated breast cancer cell growth." Experimental Cell Research. 2015 Jan 15; 330(2):358-70.

Ellsworth RE, Valente AL, Blackburn HL, Decewicz A, Deyarmin B, Mamula K, Shriver CD, Ellsworth DL. "Effect of genomic heterogeneity on breast cancer progression and metastatic spread." Society of Surgical Oncology Annual Cancer Symposium, March 25-28, 2015, Houston, TX.

Shriver CD, Hueman MT, Ellsworth RE. "Avoidance of sentinel lymph node biopsy: can molecular profiling of primary breast tumors predict lymph node status?" Society of Surgical Oncology Annual Cancer Symposium, March 25-28, 2015, Houston, TX.

Blackburn HL, Ellsworth DL, Shriver CD, Ellsworth RE. "Role of cytochrome P450 genes in breast cancer etiology and treatment: effects on estrogen biosynthesis, metabolism and response to endocrine therapy." Cancer Causes and Control. 2015 March; 26(3):319-32.

Shriver CD, Hueman MT, Ellsworth RE. "Avoidance of surgical disruption of the axillary lymph nodes: can molecular profiling of primary breast tumors predict lymph node status?" Society of Surgical Oncology Annual Meeting, March 25-28, 2015, Houston, TX.

Iida J, Elisabeth T, Bell-Loncella TB, Purazo ML, Lu Y, Dorchak J, Clancy R, Slavick J, Ferrone S, Cutler ML, Shriver CD. "Inhibition of cancer cell growth by ruthenium complexes." (submitted for publication clearance March 2015).

Constantino N, Toro AL, Shriver CD, Ellsworth EL, Ellsworth RE. "Can a diagnosis of invasive breast cancer effectively motivate patients to follow a healthy lifestyle?" (submitted for publication clearance April 2015).

Ellsworth RE, Mamula KA, Costantino NS, Deyarmin B, Kostyniak PJ, Chi LH, Shriver CD, Ellsworth DL. "Abundance and distribution of polychlorinated biphenyls (PCBs) in breast tissue." Environmental Research. 2015 April;138: 291-7.

Rummel SK, Hueman MT, Costantino N, Shriver CD, Ellsworth RE. "Association of tumor location within the breast and clinicopathological characteristics." American Association for Cancer Research, Annual Meeting, April 18-22, 2015, Philadelphia, PA.

Rummel SK, Yarina W, Toro AL, Shriver CD, Ellsworth RE. "Differential gene expression in breast tumors from African American women: separating causation from stratification." (submitted for publication clearance May 2015).

Toro AL, Constantino NS, Shriver, CD, Ellsworth, DL, Ellsworth, RE. "Effect of obesity on molecular characteristics of invasive breast tumors: gene expression analysis of 405 tumors by BMI." (submitted for publication clearance May 2015).

Chen Y, Bekhash A, Kovatich AJ, Hooke JA, Liu J, Kvecher L, Fantacone-Campbell JL, Mitchell EP, Rui H, Mural RJ, Shriver CD, Hu H. "Positive association of fibroadenomatoid change with HER2-negative invasive breast cancer: a co-occurrence study." PLoS One. 2015 June 22;10(6):e0129500.

Goodman CR, Sato T, Peck AR, Girondo MA, Yang N, Liu C, Yanac AF, Kovatich AJ, Hooke JA, Shriver CD, Mitchell EP, Hyslop T, Rui H. "Steroid induction of therapy-resistant cytokeratin-5-positive cells in estrogen receptor-positive breast cancer through a BCL6-dependent mechanism." Oncogene. 2015 June 22.

Blackburn HL, Schroeder B, Turner C, Shriver CD, Ellsworth DL, Ellsworth RE. "Management of incidental findings in the era of next-generation sequencing." Current Genomics. 2015 June; 16(3): 159-74.

Rummel SK, Hueman MT, Costantino N, Shriver CD, Ellsworth RE. "Tumor location within the breast: does tumor site have prognostic ability?" eCancermedicalscience, 2015 July 13(9):552.

Ellsworth RE, Toro AL, Blackburn HL, Decewicz A, Deyarmin B, Mamula KA, Costantino NS, Hooke JA, Shriver CD, Ellsworth DL. "Molecular heterogeneity in primary breast carcinomas and axillary lymph node metastases assessed by genomic fingerprinting analysis." Cancer Growth Metastasis. 2015 July 20(8):15-24.

Barrow TM, Barault L, Ellsworth RE, Harris HR, Binder AM, Valente AL, Shriver CD, Michels KB. "Aberrant methylation of imprinted genes is associated with negative hormone receptor status in invasive breast cancer." International Journal of Cancer. 2015 August 1:137(3): 537-47.

Valente AL, Schroeder B, Shriver CD, Henning JD, Ellsworth RE. "Chronic inflammation in cancer: the role of human viruses." Advances in Tumor Virology 2015(5):1-11.

7. **INVENTIONS, PATENTS AND LICENSES:** None

8. REPORTABLE OUTCOMES

24 August 2014 – 23 August 2015 Annual Report Numbers

Total Samples Collected
Total Blood: 2244
Total Breast: 1003
Total LN: 60
Total Other: 150

Total Patients Collected From
WRNMMC 181
Windber 65
AAMC 156

9. OTHER ACHIEVEMENTS: NONE

10. REFERENCES: NONE

11. APPENDICES:

- ATTACHMENT 1: List of personnel receiving pay from the research effort from 24 August 2014 – 23 August 2015.

Current Staff, role and percent of effort on project:

Last, First	Business Title	Level of Effort
Shriver, Craig D.	Principal Investigator	5%
Basham,Janice B	Licensed Practical Nurse	73%
Boone,Jaime J.	Senior Program Manager	70%
Campbell,Jamie Leigh	Pathologist Assist./Site Coord	66%
Ellsworth,Rachel E.	Cancer Geneticist	81%
Freeman, Benjamin	Research Assistant	64%
Hilton,Karrie R.	Assistant Head Nurse	74%
Holden,Allan	Sr.Data Management Specialist	73%
Hooke,Jeffrey A	Head of Pathology	44%
Joseph,Julie	Research Assistant II	72%
Kovatich,Albert	Scientist	48%
Miskovsky,Vicki Jones	Admin Reviewer CCC Protocols	19%
Patterson,Carol M	Medical Assistant	62%
Pereira,Dianne	Office Manager/Admin. Assist.	57%
Sakura,Sara Denman	Research Protocol Coordinator	74%
Trupp,Rebecca Saron	Nurse Navigator	46%

Wareham,Janet Andrea Yoder	Pathologists Assistant	76%
Williamson,Eric	Breast Center Administrator	71%
XXXXXXXXX	HJF IT Support	8%
Zhu,Kangmin	Assoc Dir for Epidemiology	13%
Zingmark,Rebecca N.	Histotechnologist	69%
Bronfman,Eileen T	Administrative Director	26%
Weiss,Raymond B	Physician	44%
Rigatti,Michael Kevin	Research Assistant	7%
Smith,Stephanie R	Research Nurse	11%
Vilakazi,Patricia N.	Biomedical Informatics Coord.	35%
Davis,Herma Elaine	Sr. Data Manager	62%

- ATTACHMENT 2: Expenditures from 24 August 2014 – 23 August 2015.

Total Cumulative Expenditure
for award W81XWH-12-2-0050
24 August 2014 – 23 August 2015

Personnel:	2,227,749.70
Consultants:	0.00
Equipment:	118.25
Supplies:	87,532.69
Domestic Travel:	39,125.46
Foreign Travel:	1,729.00
Rent:	33,415.81
Other Direct Cost:	682,797.99
Sub award:	3,564,395.62
Total Direct Cost:	**6,633,406.48**
Indirect Cost:	481,105.86
Fee:	-
Total Program Cost:	**$7,114,512.34**

- ATTACHMENT 3 : Agenda from the BCTR-CoE one day offsite meeting on 24 July 2015 at the Uniformed Services University of the Health Sciences

Clinical Breast Care Project (CBCP) Retreat

USUHS, Sanford Auditorium
Friday, July 24, 2015

7:30 – 8:00 AM	**Registration and Continental Breakfast**	
8:00 – 8:05 AM	**Welcome and Announcements**	**Jaime Boone, MBA**
8:05 – 8:20 AM	**Opening Remarks**	**Craig D. Shriver, MD, FACS**
8:20 – 8:35 AM	**Greetings from WRI**	**Tom Kurtz, President WRI**
8:35 – 8:55 AM	**CBCP Research Update**	**Hai Hu, PhD**
8:55 – 9:15 AM	**Tissue Bank**	**Stella Somiari, PhD**
9:15 – 9:35 AM	**Biomedical Informatics Infrastructure**	**Leonid Kvecher, MS**
9:35 – 9:55 AM	**Translational Breast Research Update**	**Rachel Ellsworth, PhD**
9:55 – 10:05 AM	**Break**	
10:05 – 10:25 AM	**Pathology Update**	**Al Kovatich, Scientist**
10:25 – 10:45 AM	**Research Update**	**Juliana Slavik, MS for George Iida**
10:45 – 11:05 AM	**Genetics Update**	**Raymond Weiss, MD**
11:05 – 11:15 AM	**Nurse Navigators**	**Becky Trupp, RN**
11:15 – 11:30 AM	**AAMC Breast Center**	**Dr. Lorrain Tafra, MD**
11:30 – 11:45 AM	**JMBCC Breast Center**	**Dr. Deborah Sims, MD**
11:45 – 12:00 PM	**Discussion Wrap Up of Morning**	**Craig D. Shriver, MD**
12:00 – 12:15 PM	**Group Photo**	**Outdoor Quad Area**
12:15 – 1:15 PM	**Lunch on Your Own**	**USUHS Cafeteria**
1:15 – 1:45 PM	**Status of CBCP protocols / New CRF**	**Sara Sakura, PsyD, CCRP** **Leigh Campbell, M.S., P.A. (ASCP)**
1:45 – 3:45 PM	**Discussion / Visioning for Future**	**Craig D. Shriver, MD / Group**
3:45 – 4:00 PM	**Concluding Remarks**	**Craig D. Shriver, MD**